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Tract Based Spatial Statistic reveals no differences in white matter microstructural organisation between carriers and non-carriers of the ApoE ε4 and ε2 alleles in young healthy adolescents

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Abstract

The Apolipoprotein E (ApoE) ε4 allele is the best established genetic risk factor for Alzheimer's disease (AD) and has been previously associated with alterations in structural gray matter and changes in functional brain activity in healthy middle-aged individuals and older non-demented subjects. In order to determine the neural mechanism by which ApoE polymorphisms affect white matter (WM) structure, we investigated the diffusion characteristics of white matter tracts in carriers and non-carriers of the ApoE ε4 and ε2 alleles using an unbiased whole brain analysis technique (Tract Based Spatial Statistics (TBSS)) in a healthy young adolescent (14years) cohort. A large sample of healthy young adolescents (n=575) were selected from the European neuroimaging-genetics IMAGEN study with available ApoE status and accompanying diffusion imaging data. MR Diffusion data was acquired on 3T systems using 32 diffusion-weighted (DW) directions and 4 non-DW volumes (b-value = 1300 s/mm^2 and isotropic resolution of 2.4x2.4x2.4 mm). No significant differences in WM structure were found in diffusion indices between carriers and non-carriers of the ApoE ε4 and ε2 alleles, and dose-dependent effects of these variants were not established, suggesting that differences in WM structure are not modulated by the ApoE polymorphism. In conclusion, our results suggest that microstructural properties of WM structure are not associated with the ApoE ε4 and ε2 alleles in young adolescence, suggesting that the neural effects of these variants are not evident in 14-year olds and may only develop later in life.

Keywords

Apolipoprotein E, Diffusion Tensor Imaging, DTI, TBSS, magnetic resonance imaging, young healthy adolescents.

Introduction

The Apolipoprotein E (ApoE) gene is a low density glycoprotein which has a key metabolic role in facilitating the transport and delivery of lipids such as cholesterol from one cell type to another [1,2]. A key understanding in its function beyond normal lipoprotein metabolism has led to the emergence of its role in modulating neuronal repair, brain plasticity and axonal myelination. In particular, an isoform of ApoE, ApoE ε4, has been associated with an increased risk of developing sporadic Alzheimer's disease (AD) [3–5], whereas the ApoE ε2 isoform has been suggested to confer a protective effect against the disease [6].

Neuroimaging studies of different ApoE gene polymorphisms, particularly the ApoE ε4 allele, have provided evidence of its association with changes in gray matter structure in AD patients, subjects with Mild Cognitive Impairment (MCI), and non-demented elderly controls [7–10]. A majority of these findings have shown that ApoE ε4 carriers reflect greater rates of atrophy in the hippocampus and entorhinal cortex - key regions known to be affected in early AD. Further, similar neuroanatomic effects of the ApoE ε4 allele have been observed in young healthy adults (20-35 years), suggesting that possession of the ε4 allele may also be associated with a neural endophenotype that is more susceptible to age-related neurodegeneration in later life [11–13]. The emergence of these findings has led to an increased interest in exploring even earlier brain differences associated with the ε4 allele. As a result, some have postulated that the neuroanatomic effect of the allele on brain phenotypes associated with AD risk could even precede birth [14].

Functional MRI studies performed in healthy adults both at rest and during a memory-encoding paradigm also reveal altered patterns of brain activation in healthy

ApoE ε4 carrier subjects [15,16]. However, findings on the functional effects of the ApoE ε4 allele are mixed, and evidence for both increased and decreased brain activation have been previously reported [17].

Diffusion tensor imaging (DTI) tractography is a technique that is being increasingly used to assess white matter changes in the brain by quantifying the microstructural density and orientation of axonal bundles connecting different regions of the brain [18,19]. Understanding the neural mechanism by which the ApoE polymorphism influences WM structure is crucial, particularly because ApoE plays a major role in distributing essential lipids that contribute to the development of myelin sheath [20,21].

Previous DTI studies of older healthy ApoE ε4 carriers, have found reduced fractional anisotropy (FA) in the splenium of the corpus collosum [22], the cingulum bundle [23,24], and parahippocampal gyrus [25]. A recent study into younger ApoE ε4 carriers reported non-significant differences in WM structure, but found subtle differences within the prefrontal cortex between carriers and non-carriers [26]. Carriers of the ApoE ε2 allele are believed to have a decreased risk of developing AD [6], though the basis for this decreased vulnerability to the disease remains equivocal.

Myelination of intra-cortical fibers progresses gradually from birth to adulthood [27], and it is likely that asynchronous brain maturation processes during adolescence, particularly synaptic pruning and changes in axon calibre, may lead to a WM network that is more susceptible to structural damage in later life [28]. Using a large sample of young healthy adolescents from the European IMAGEN study, we assessed the

diffusion characteristics of ApoE ε4, ε3 and ε2 carriers to determine if WM microstructure properties are modulated by the ApoE polymorphism.

Materials and Method

Participants

Data analysed for this study were obtained from healthy 14-year olds from the IMAGEN project, a European multi-centre neuroimaging-genetics study in adolescence. A total of 575 healthy adolescents were selected from the study with available diffusion imaging and ApoE genotype data. Written informed consent was obtained from all participants and their legal guardians, further information on recruitment procedures has been previously provided [29]. Participants completed an extensive battery of neuropsychological, clinical, personality and drug use assessments.

For each subject, blood samples were collected for DNA analysis and extraction. Samples were subsequently genotyped using the Illumina Quad 610 and 660 arrays (Illumina, San Diego, CA, USA). Two ApoE single nucleotide polymorphisms, rs429358 (T, C) and rs7412 (C, T) were used to identify 3 allelic variants of ApoE (ε2, ε3, and ε4) in order to define a subject's genotype. Eight subjects with an ApoE ε4 and ε2 genotype were excluded from the main analysis as previous studies have proposed an opposing neuroanatomic effect of the ApoE ε4 and ε2 alleles. The demographic characteristics of our sample are described in Table 1.

Image Acquisition

575 subjects (mean age 14.43 ± 0.47 years) from 5 imaging centers with compatible DTI acquisition protocols and with ApoE genotype information available were selected. A standardised imaging protocol was used to ensure homogeneity in data acquisition across different scanners [29] . MR Diffusion data was acquired on 3T

147 systems with the following parameters: TE=104ms, TR=15000ms, matrix size
148 128x128, number of slices=60 and isotropic resolution of 2.4x2.4x2.4mm. Diffusion
149 data was acquired with a b-value of 1300 s/mm^2 along 32 non-collinear diffusion
150 gradient directions and 4 non-diffusion weighted volumes. More details are available
151 in Schumann et al 2010 [29].

152 Image Analysis

153 Diffusion data was processed using ExploreDTI [30]. Data was first preprocessed to
154 correct for eddy current distortions and head motion. For each subject the b-matrix
155 was reoriented to provide a more accurate estimate of tensor orientations. Diffusion
156 tensor was estimated using RESTORE [31] an automatic and iterative approach for
157 the automatic rejection of data outliers. Finally, Fractional Anisotropy (FA), Mean
158 Diffusivity (MD) and Axial and Radial Diffusivity maps were generated. Diffusion
159 imaging data quality was assessed by automatically identifying outliers using
160 residual maps of the tensor fitting and by visually inspecting each subject for major
161 anatomical abnormalities (21 subjects were excluded from the analysis).

162 Voxel-wise statistical analysis on FA, MD, axial and radial diffusivity maps was
163 carried out on the remaining 554 subjects using Tract-Based Spatial Statistics
164 (TBSS) [32]. All subject's FA data were registered to the standard MNI space using a
165 study specific template generated from 50 randomly selected subjects. To exclude
166 low anisotropic regions, an FA threshold equal to 0.3 was selected to generate the
167 FA skeleton. A more commonly used FA threshold of 0.2 was also applied to
168 generate the WM skeleton, yet no significant effects of ApoE genotype from the
169 TBSS analysis were found. Consequently, we applied a FA threshold equal to 0.3 in
170 order to restrict the analysis to more anisotropic regions with less significant inter-

subject variability and partial volume effects with grey matter. In other words, we excluded peripheral tracts that we believe did not assume good tract correspondence and WM alignment across our subject sample.

Cognitive Assessment

The Block Design and Matrix Reasoning subtests of the Wechsler Intelligence Scale for Children-Fourth Edition [33] were computed to generate a Perceptual Reasoning Index and assess nonverbal intelligence (nonverbal intelligence quotient (IQ)). The Similarities and Vocabulary subtests were computed to generate a Verbal Comprehension Index measuring verbal concept formation, that is, the subjects' ability to verbally reason (referred to as verbal IQ).

Statistical Analysis

After quality control, 546 subjects from the IMAGEN cohort were used to perform non-parametric two sample t-tests (5000 permutations) using a generalised linear model on each diffusion index. Comparisons between carriers and non-carriers of the ApoE ε4 and ε2 alleles were performed on the diffusion measures and differences between ApoE ε4 and ε2 carriers were also investigated. Variables of gender, scan centre, verbal and nonverbal IQ were included as covariates in the analysis. The R statistical software environment, version 3.1.0, was used to compare basic demographic statistics by ApoE genotype [34].

Results

Demographic Characteristics

Healthy adolescents possessing either the ApoE ε4 or ε2 alleles did not significantly differ in terms of demographics verbal IQ and nonverbal IQ in relation to ApoE ε3/ε3 homozygote carriers. Furthermore, measures of cognitive functioning also did not significantly differ between the groups (Table 1).

Diffusion Imaging TBSS

In the TBSS analysis, we firstly compared healthy adolescents with at least one ApoE ε4 allele to non-carriers of the ε4 allele (ε3/ε3 homozygotes and ε3/ε2 carriers). The analysis revealed no significant differences between carriers and non-carriers of ApoE ε4 for any index of diffusion (FA, MD, axial and radial diffusivity (respectively AD and RD)) at a p-value threshold of $\alpha = 0.05$. Similarly, no significant effect of the ApoE ε2 genotype was found in ε2 carriers and non-carriers of the allele (ε3/ε3 homozygotes and ε3/ε4 carriers). Furthermore, comparisons between ε4 and ε2 carriers also yielded no significant findings in the TBSS analysis. Although our cohort had a small number of ε4 homozygote (ε4/ε4) carriers (n=5) and ε2 homozygote (ε2/ ε2) carriers (n=2), there was no evidence to suggest any ApoE ε4 or ApoE ε2 dose-dependent effects on WM structure.

Discussion

To our knowledge, this is the first study to investigate the diffusion characteristics of WM microstructure in relation to ApoE ε4 and ε2 genotype in young healthy adolescents. Firstly, we investigated the neural effect of the ApoE ε4 genotype on WM structure and TBSS analysis revealed no significant differences in diffusion indices including FA, MD, RD, and AD between ε4 carriers and non-carriers. Previously, we were able to show that no significant differences in hippocampal volume were present between ApoE ε4 carriers and non-carriers in a wider group of these young subjects [35]. This finding, along with those from previous structural imaging studies in older adults, led us to postulate that WM changes could precede alterations in grey matter – possibly affecting key allocortical fiber pathways that may reflect ApoE-driven vulnerabilities in the WM network. For instance, Heise and colleagues [36] were able to demonstrate that the ApoE ε4 allele modulated the WM structure of young adult carriers despite no evidence of changes in grey matter structure. Yet, diffusion-based imaging studies measuring regional patterns of WM changes in AD and older healthy subjects have reported mixed findings, with some providing support to the hypothesis of WM degeneration of later-myelinating fibers [37,38] and others proposing WM damage to the medial temporal lobe and to prefrontal regions responsible for sustaining memory function [39,40]. In particular, a number of DTI studies in AD patients and MCI subjects have reported reduced FA values in multiple regions, however, areas with more consistent findings include the hippocampal formation [39], parahippocampal gyrus [41] ,and the cingulum bundle [23,42].

In contrast, healthy young adult ApoE ε4 carriers studies have also shown reduced FA values in similar regions known to be affected in patients with AD[43,44], with

some in the absence of brain fibrillar amyloid plaque deposition [45]. Amyloid β dysregulation is believed to begin several decades before memory impairment in sporadic AD. Many studies have since proposed that amyloid biomarkers are the first to become abnormal in the pathophysiological cascade of the disease. However, recent structural and functional studies have suggested that tau-mediated neurodegeneration may precede amyloid pathology in individuals possessing the ApoE ε4 genotype [46,47]. These findings in young adults have since prompted an increased interest to address brain changes associated with the ApoE ε4 allele in infant brain development. In particular, Knickmeyer and colleagues [48] examined the effect of psychiatric risk variants, including ApoE ε4 genotype, in prenatal brain development and discovered reduced temporal cortex volumes in ε4 carriers. However, as the authors of this study had acknowledged, the study sample was enriched to contain infants with a parental history of psychiatric and neurological illness. Building on the findings from this study, Dean and colleagues [49] compared measurements of white matter and gray matter volume in typically-developing infants with no family history or AD or psychiatric illness. Specifically amongst ApoE ε4 carriers, white matter myelin content and gray matter volumes were reduced in the precuneus, lateral temporal and middle cingulate – regions of the cortex affected in late-onset AD. Despite the intriguing result, multiple regions were compared and most results did not survive multiple comparisons testing, and the number of ε4 carriers was substantially higher than the general population.

In adolescence, brain development represents a major transition in neurobiological processes which influence specific maturational changes in brain structure and function. These changes usually optimise the brain for underlying cognitive and behavioural changes that manifest at the time of puberty onset, but could also confer

a neural vulnerability to certain forms of psychiatric illness later in life. It is therefore likely that dynamic stages of individual brain development, such as differences in the myelination of intra-cortical fibers [50], change in axon calibre [28], and synaptic density [27] could contribute to a variability in WM networks of adolescents. As a result, a decrease in statistical sensitivity could be attributed to one or some of these potential factors. Nonetheless, our finding that the ApoE ε4 polymorphism does not modulate WM structure suggests that its effect is not detectable in young adolescence. Instead, a neuroanatomic effect of ApoE ε4 allele may only become apparent in later adulthood, particularly when age-related changes in WM structure begin to gradually decline [51].On the other hand, no genotypic effect of the ApoE ε2 allele was found on any diffusion indices to suggest a protective effect on WM structure. To date, only one study, by Chiang and colleagues [52] found higher FA values in the posterior cingulate and anterior corpus collosum, suggesting those harbouring an ApoE ε2 genotype had more robust WM integrity, and consequently, decreased vulnerability to AD pathogenesis. In the past, several studies have proposed to explain the effects of this allele, including cellular models advocating its role in blocking the aggregation of amyloid β peptides [6,53], and studies reporting thicker cortices in carrier groups [11,54]. Yet, the neural mechanism of its putative protective effect still remains unclear.

Our findings should be noted in light of some limitations. Firstly, although TBBS, a localised statistical method, aims to alleviate problems in residual misalignment, partial gray matter volume contamination cannot be excluded, especially when the width of the tract is smaller than the original voxel size [32]. However, thresholding mean FA values between 0.2 and 0.3 on the WM skeleton has been known to successfully exclude voxels that are primarily grey matter or CSF [55]. Although the

283 procedures used here may be superior to standard registration procedures, the
284 potential exists to apply more advanced methods that can better quantify the
285 complexity of white matter changes in the brain using full tensor information [56] and
286 tract-specific indices of diffusion [57]. Given our observation of no ApoE genotypic
287 effect on WM structure, it would be interesting for future longitudinal studies to
288 address when ApoE modulated changes in the WM network begin to manifest and if
289 they vary with age. Furthermore, since adolescence is a particurarly active
290 neurodevelopmental period we cannot exclude the possibility of a reduced ApoE
291 penetrance in this age. Nevertheless, previous studies in younger asymptomatic ε4
292 carriers have shown that ApoE plays a fundamental role in modulating brain function
293 in the absence of any differences in brain volume[16]. Future studies examining the
294 pattern of brain activity both at rest and during a memory encoding paradigm in
295 these individuals will help us understand if genotype-dependent changes in brain
296 function manifest before structural differences.

297 Overall, this study demonstrated that individuals possessing the ApoE ε4 and ε2
298 alleles do not have different WM microstructural properties at a young age (14
299 years). This suggests that WM structure in young adolescence is not modulated by
300 the ApoE polymorphism, but may reflect distinct genotype-dependent vulnerabilities
301 in its structure later during adulthood.

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Table 1

Demographic and cognitive characteristics of ApoE ε3 carriers, ε4 carriers and ε2 carriers from the IMAGEN study cohort.

	ε3 carriers	**ε4 carriers (*n* = 119)**		**ε2 carriers (*n* = 55)**		
	ε3/ε3 (n=372)	**ε3/ε4 (n=114)**	**ε4/ε4 (n=5)**	**ε2/ε3 (n=53)**	**ε2/ε2 (n=2)**	***p*-value**
Age (Years)	14.4 ± 0.5	14.4 ± 0.5	14.6 ± 0.5	14.4 ± 0.5	14.3 ± 0.1	0.975
Gender (Male/Female)	204/168	68/46	2/3	27/26	0/2	0.662
Handedness (Right/Left/Both)	321/46/5	106/8/0	4/1/0	48/4/1	2/0/0	0.477
Verbal IQ	114.0 ± 14.8	114.0 ± 14.7	116.8 ± 20.1	111.5 ± 14.1	125.5 ± 20.5	0.638
Nonverbal IQ	109.4 ± 13.5	110.4 ± 13.9	103.8 ± 8.3	109.5 ± 12.5	96.0 ± 5.7	0.821
CANTAB SWM	30.8 ± 5.4	30.7 ± 5.7	29.0 ± 6.0	30.1 ± 5.0	33.0 ± 4.2	0.727
BMI	20.6 ± 3.4	20.2 ± 2.5	21.0 ± 2.3	20.3 ± 3.2	17.3 ± 2.4	0.446
WISC Block Design	52.1 ± 8.0	52.5 ± 8.5	49.8 ± 5.0	51.7 ± 7.4	35.0 ± 5.7	0.598
WISC Matrix Reasoning	27.1 ± 3.7	27.4 ± 3.7	26.0 ± 5.2	27.4 ± 3.8	26.0 ± 1.4	0.790
WISC Similarities	31.3 ± 4.8	31.6 ± 5.0	33.0 ± 7.5	30.8 ± 4.2	33.5 ± 7.8	0.672
WISC Vocabulary	51.1 ± 7.5	51.1 ± 7.6	51.2 ± 9.4	49.9 ± 7.6	60.5 ± 5.0	0.726

Values represent Mean ± Standard Deviation.

A p-value of 0.05 was considered significant for all tests.

Continuous variables were inspected using analysis of variance (ANOVA) with post-hoc Bonferroni tests and categorical variables (Gender and Handedness) were inspected using fisher exact tests.

Abbreviations: Verbal IQ = verbal intelligence scale, Nonverbal IQ = nonverbal intelligence scale, CANTAB SWM = CANTAB Spatial Working Memory, BMI = Body Mass Index, WISC = Wechsler Intelligence Scale for Children.

All ApoE ε4/ε2 allele carriers were excluded from the study.